Indonesian Journal of Biotechnology, December (1999) 326 - 329

Analysis of bovine herpesvirus-1 antibodies by enzyme immunoassay in Indonesian cattle

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Abstract

Enzyme-linked immunosorbent assay (ELISA) (HerdCheck: Anti-IBR) of 231 sera samples, all originated from Indonesian Holstein Friesian crossbred cattle with predetermined history of freedom from BHV-1 vaccination revealed that the ELISA was able to detect specific BHV-1 antibodies, estimated by testing 121, 65 and 45 sera showed the antibody positive sera of 11.6, 24.6 and 22.2%, respectively. Because no vaccination is allowed, it appears that the ELISA posive cattle may have been infected with IBR field strains. The ELISA for the routine detection of antibodies against BHV-1 in Indonesian cattle is recommended as screening for natural exposure to IBR infection in order to control and eradicate further risk of disease transmission.

Keywords: *ELISA (HerdCheck: Anti-IBR)-IBR-Holstein Friesian*

Introduction

Infectious bovine rhinotracheitis (IBR) is a highly contagious infectious disease that is caused by bovine herpesvirus-1 (BHV-1) (Donkersgoed and Babiuk, 1991). The BHV-1 is the most important herpesvirus of cattle (Madin *et al.*, 1956) and is recognized as a serious disease threat to beef and dairy cattle worldwide (Smith *et al.*, 1995). The virus can cause respiratory disease, conjunctivitis, the genital diseases-infectious pustular vulvovaginitis in females and infectious pustular balanoposthitis in males, abortions, enteritis, encephalitis and fatal systemic infections (Gibbs and Rweyemamu, 1977; Ludwig, 1983; Wyler *et al.*, 1989). The clinical signs that are observed depend on a number of factors (Yates, 1982). Most recently, attention has been drawn to the BHV-1 infection, complicated by infection of non-cytopathic bovine viral diarrhea virus (NCP-BVDV). A complication of NCP-BVDV associated with BHV-1 infection is the ability of the NCP-BVDV to cause a persistent infection (Wasito and Wuryastuti, 1996; Wuryastuti and Wasito, 1997; Wuryastuti and Wasito, 1998).

Different approach(es) have been used to make a diagnosis for BHV-1 infection in cattle (Donkersgoed and Babiuk, 1991; Kramps *et al.*, 1993; Smith *et al.*, 1995). In the present study, a commercial enzyme-linked immunosorbent assay (ELISA) for BHV-1 specific antibody detection in serum has been applied using bovine sera of Indonesian Holstein Friesian crossbred cattle.

Materials and Methods

Collection of bovine sera samples

Clotted blood were collected from a total of 231 dairy cattle of all ages (2 months to 6 years) and had never been vaccinated against infectious bovine rhinotracheitis virus. None of the cattle bled had clinical signs of illness. The samples were collected from both commercial and traditional farms, in 2 provinces: East Java (Batu/Malang) and Central Java (Yogyakarta and Baturaden/Purwokerto). Of these samples, all originated from Holstein Friesian crossbred cattle.

Blood samples were taken from the jugular vein and collected into sterile polystyrene tubes. Te tubes were immediately placed in a precooled styrofoam box in order to avoid exposure to elevated outside temperatures. Upon arrival at the laboratory, the blood samples were centrifuged at 600 g for 10 minutes at room temperature. The sera were collected into 1 ml Eppendorf tubes and stored at -70° C until tested.

Enzyme-linked immunosorbent assay (ELISA)-infectious bovine rhinotracheitis virus (IBR) antibody test kit

In the present seroepidemiological study to determine the prevalence of antibody to IBR, the sera of bovine origin were tested in duplicate on two different occasions by all an ELISA test kit (HerdCheck: Anti-IBR) provided by Dr. Liauw at IDEXX Laboratories Inc., Maine, U.S.A. This test is designed to detect the presence of antibody to IBR in bovine serum or milk.

Sera samples to be tested were diluted forty-fold (1:40) with sample diluent (e.g. by diluting 10 ml of serum, with 390 µl of sample diluent). Each 100 µl diluted sample was then added to a well coated with IBR antigen and also to a well coated with normal host cell (NHC) antigen. The immobilized NHC antigen coated wells were used to determine whether immunoglobulins against tissue culture components, potentially present in vaccines, are contributing to the test results. The diluted samples were incubated for 90 minutes at room temperature (20-25° C). Following four times washes with approximately 300 µl of wash solution to remove unbound material, 100 µl of anti-bovine IgG:Horseradish Peroxidase (HRPO) conjugate was added into each well. The microwell plates were incubated for 30 minutes at room temperature (20-25° C). Next, unbound conjugate was removed in four times washes with approximately 300 µl of wash solution. The chromogen/substrate solution was then added and the plates were incubated for 15 minutes at room temperature (20-25° C). At the end of this incubation periode, 100 microliter of stop solution was added to each well. Color development in samples and controls was quantitated spectrophotometrically at a wavelength of 650 nanometers.

Results and Discussions

Bovine herpesvirus 1 (BHV-1) is the cause of infectious bovine rhinotracheitis (IBR), a severe upper respiratory tract disease of cattle (Miller *et al.*, 1995). In addition, some virus strains may induce genital, neurologic or generalized systemic disease (Gibbs and Rweyemamu, 1977; Straub, 1990; Straub, 1991). Some of these strains may cause abortion in pregnant cattle after transplacental infection and death of the fetus (Straub, 1991; Miller *et al.*, 1995). Consequently, BHV-1 infections are responsible for severe economic losses (Kramps *et al.*, 1993). Although clinical findings may be highly suggestive of IBR, no real pathopneumonic signs are restricted to IBR. Therefore, laboratory confirmation is necessary in order

to definitely identify BHV-1 infection (Van Donkersgoed and Babiuk, 1991). Confirmation of exposure to BHV-1 via natural infection is facilitated by a measurement of antibody in serum or milk. The enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against BHV-1 in cattle has been shown to correlate with the virus neutralization test (VN), although the ELISA can be more sensitive (Herring *et al.*, 1980; Riegel *et al.*, 1987; Anonymous, 1996).

The present study was done to determine the prevalence of antibodies to BHV-1 in both commercial and traditional cattle population of Batu/Malang, East Java, and Yogyakarta and Baturaden/Purwokerto, Central Java. In the present study, to confirm that cattle expressed antibodies to infectious bovine rhinotracheitis virus (IBR), a total of 231 sera from all cattle were tested for bovine herpesvirus-1 (BHV-1) by use of an enzyme-linked immunosorbent assay (ELISA). This testing was done in the laboratory of Immunochemistry/Chemistry, Inter University Center for Biotechnology, Gadjah Mada University, Yogyakarta, Indonesia.

In the Batu, Malang area of East Java, 14 out of 121 samples from Holstein Friesian crossbred cattle tested, were positive for anti-BHV-1 antibodies. This translates into a prevalence of 11.6%. A total of 65 sera from Holstein Friesian crossbred cattle in Kulonprogo/Yogyakarta, Central Java were also tested by the ELISA. Sixteen of these sera tested, were positive, for a prevalence 24.6%. Whereas, BHV-1 antibodies were detected from 10 out of 45 sera collected from Holstein Friesian cattle in

Baturaden/Purwokerto, Central Java, for a prevalence of 22.2% (Table 1). Using this ELISA test, we were able to confirm the presence of a lot amounts of specific BHV-1 antibodies in most sera tested as indicated by a development of strong blue colour observed in wells coated with IBR antigen (Fig. 1). Because no vaccination is allowed, the ELISA test confirmed serologically those cattle infected with IBR field strains, as required for IBR control and eradication program. The data presented suggested that the anti-BHV-1 antibody ELISA test could be useful in screening for natural exposure to IBR infection.

Table 1. Prevalence of antibodies to bovine herpesvirus-1 (BHV-1) in Indonesian cattle, as determined by a commercial enzyme-linked immunosorbent assay (ELISA)

No.	Origin of the sera	Cattle breed	No. of sera	ELISA +	ELISA -
1.	Malang	Holstein Friesian	121	14	107
2.	Yogyakarta	Holstein Friesian	65	16	49
3.	Baturaden	Holstein Friesian	45	10	35

Figure 1. Anti-BHV-1 antibody ELISA on sera samples from Holstein Friesian crossbred cattle in Batu/Malang, East Java. Notice strong color developments as blue ELISA-positive BHV-1 antibodies in wells coated IBR antigen.

Acknowledgement

We thank Prof. Dr. Roger K. Maes, the Adviser for thoughtful advice during this study. The IBR ELISA test kit provided by Dr. Liauw at IDEXX Laboratories is greatly appreciated.

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